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TITLE: Identification of Ovarian Cancer Susceptibility Genes Involved in Stromal-Epithlial Interactions

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14. ABSTRACT

No results from this study are available yet because the first stage of genotyping has not been completed, but we have selected 1536 tagging, non-synonymous and miRNA binding site SNPs in 174 candidate genes, and typing of them is underway in 900 serous invasive ovarian cancer cases and 1200 controls.

15. SUBJECT TERMS
risk, ovarian cancer, polymorphisms, genes, stromal-epithelial interactions

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INTRODUCTION

We propose that subtle variation in the expression or function of genes expressed as a consequence of interactions between ovarian cancer cells and the host micro-environment could contribute to susceptibility to ovarian cancer. This idea is novel because this class of genes has not previously been tested for a role in ovarian cancer susceptibility. Our approach, and our choice of candidate genes, is based on extensive preliminary data we have accumulated from co-culture of fibroblast and epithelial ovarian cells. Our original aim was to identify all non-synonymous coding and putative promoter SNPs in 60 candidate genes highlighted by our analysis of cross talk between fibroblast and epithelial elements of ovarian tumors, as well as a set of haplotype tagging SNPs in 20 of these co-culture regulated genes which are altered in expression in serous tumours, compared with normal ovarian surface epithelial cells. However, since the start of this project we have acquired an Illumina Bead Station and so can genotype 1536 SNPs in the first stage, allowing us to genotype potentially functional as well as tagging SNPs in all the genes of interest. We then planned to genotype the SNPs in 900 cases with serous ovarian adenocarcinoma, and 1300 controls, and to assess the role of these SNPs and their haplotypes in serous invasive ovarian adenocarcinoma risk. This will be followed by independent validation of positive associations we find using a replication set of at least 2,100 cases with serous ovarian adenocarcinoma and 3,600 controls. Finally, we will look for the putative functional SNPs in these genes, and evaluate their function *in vitro*.

BODY

This statement of work was altered in December 2006, and to my knowledge it was approved in January 2006 when I last emailed Theresa Miller about it to confirm that our grant office was aware that I had requested permission to change my statement of work. The new SOW was submitted because we have changed genotyping platforms which allows us to genotype many more SNPs, but with an altered the time frame. The tasks below are from the new SOW.

Task 1. *In silico* identification of SNPs in candidate genes (months 1-9)

1. *identification of 174 candidate genes involved in cross talk*

The original application proposed genotyping of candidate genes based on a series of *in vitro* experiments involving co-culture of ovarian epithelial and theca fibroblast cells. The genes were further prioritized based on elevated expression in two published ovarian cancer expression profiling studies, as well as an in house expression profile and we then generated a list of 255 candidate genes of interest.

2. *identification of 1536 tagging SNPs, nsSNPs and SNPs in putative microRNA binding sites in these 174 genes*

With Drs Ellen Goode and David Rider at the Mayo Clinic, and Illumina Inc., we then generated a list of SNPs within 5 kb of these 255 genes (58,114 SNPs in total). We then used the binning algorithm of LDSelect to identify 4567 tagSNPs among these, with (r2) $\geq$ 0.8 and minor allele frequencies (MAFs) > 0.05, using data from a variety of sources. Then we prioritized the list to 166 genes based on known function and the number of bins in each gene (excluding genes with a large number of bins), in an attempt to reduce the list to ~1500 SNPs.

We then requested from Illumina Inc the design scores for all SNPs within 5kb of these 166 genes and picked the best tagSNP in each bin (or two tagSNPs if there are >10 tagging SNPs in a bin and none had an optimal design score).We also used www.patrocles.org to identify SNPs (with MAFs $\geq$ 0.05) in microRNA binding sites within these genes, and added nsSNPs (with MAFs $\geq$ 0.05) from the public databases to the potential SNP list. This identified 170 miRNA binding sites and nsSNPs with Illumina design scores > 0.6 in these 166 genes. In total this gave 1410 tagSNPs, miRNA binding site SNPs and nsSNPs, and so the list was supplemented by tag and supplemental SNPs in another 12 candidate genes, bringing the number of genes represented in the final list to 174, in which there were 1509 SNPs meeting the above criteria (some of the original 166 candidate genes had no appropriate SNPs in them). In order to reach the final total of 1536 SNPs for the Illumina OPA, the MAF of the supplemental SNPs was dropped to 0.01. The final list of 1536 SNPs included 106 supplemental SNPs and 1430 tagSNPs. The Illumina OPA for these 1536 SNPs was ordered in December 2006, and received early in February 2007.

Task 2. Genotyping of the AOCS/ACS test set for 1536 SNPs using the Illumina Goldengate Assay (months 10-15)

1. *preparation of plates of serous invasive ovarian cancer cases and controls*

While the design of the Illumina OPA was underway we completed the extraction and QC of 1350 case and 1100 controls DNAs from the Australian Ovarian Cancer Study (AOCS), and the preparation of plates for Goldengate genotyping is underway using cases and controls from both the AOCS and the Australian Cancer Study. The plates have been designed, with 900 serous invasive cases and 1200 controls, and we are making them in sets of four so that we can genotype them and perform quality control evaluation before we make the next set. Transition to the new platform is going well, but we are being conservative in our approach, and hence it is taking a little more time that expected.

2. *genotyping of 900 cases and 1200 controls for 1536 SNPs using the Illumina Goldengate Assay*

So far 96 samples have been genotyped by Goldengate and the preliminary quality control evaluation suggests that the call rates are excellent, that the number of SNPs that cannot be genotyped is low, and that the duplicate concordance rates are very high. However, more extensive QC cannot be undertaken until additional plates have been genotyped, because the Illumina algorithms cannot perform adequately on only 96 DNA samples. Depending on our access to the Institute's Bead Station, we anticipate that this genotyping will be completed by the end of May.

Task 3. **Genotyping of the AOCS/ACS test set for additional SNPs by Mass Array and statistical analysis of test set (months 16-21)**

1. *genotyping 900 cases and 1200 controls by Mass Array for 70 SNPs that were not amenable to Illumina genotyping in 13 key genes using 30-plexes*
2. *statistical analysis of test set*

Task 4. **Genotyping of the replication set and statistical analysis of replication set (months 22-32)**

1. *genotyping 1200 cases and 3600 controls by Mass Array for 45-60 SNPs in 30-plexes, significantly associated with ovarian cancer risk in the test set (P< 0.001)*
2. *statistical analysis*

Task 5. **DHPLC to identify putative functional SNPs in genes associated with serous invasive ovarian cancer in both the test and replication set (months 25-35)**

1. *design of DHPLC primers*
2. *DHPLC of coding and conserved regulatory regions of ~5 genes in 94 moderate familial risk ovarian cancer cases*

Task 6. **Functional evaluation of putative rSNPs (months 28-36)**

Task 7. **Manuscript preparation (months 32-)**

KEY RESEARCH ACCOMPLISHMENTS

The major achievement today has been the ability to use the Illumina Bead Station which allows us to genotype 1536 SNPs in our first stage, instead of only 440 SNPs as originally estimated, and so provide full coverage of all 174 candidate genes.

REPORTABLE OUTCOMES

Abstract submitted to the upcoming AACR meeting on 'Approaches to complex pathways in molecular epidemiology' in Albuquerque in May 2007.

CONCLUSION

Progress is satisfactory, but there are no conclusions to report yet.

REFERENCES

None

APPENDICES

None